Original Research Article

Green synthesis and characterization of Silver Nanoparticles synthesized using *Piper longum* and its antioxidant activity

Running title: Synthesize of *Piper longum* based silver nanoparticle and to analyze its antioxidant activity.

Abstract:Introduction should be concised, add objective after introduction in abstract

Introduction: *Piper longum*, a traditional plant used for regenerative medicine commonly known as the long pepper, is used to treat many pathological conditions. *Piper longum* is used as a source for obtaining the production of various nanoparticles and testing their efficacy. *Piper longum* has many beneficial activities, such as antifungal, anti-amoebic, anti-asthmatic, anti-cancer, anti-oxidant, anti-inflammatory, antidepressants activities. Silver nanoparticles are widely and commonly used for its antimicrobial activity against various microorganisms. Our study aimed to synthesize *Piper longum* based silver nanoparticles and to investigate its anti-oxidant property.

Material and methods: *Piper longum* plant extract was prepared and silver nanoparticles extract was synthesized. DPPH assay was then performed to assess the free radical scavenging activity of the *Piper longum* silver nanoparticle extract. The further analysis was that the formation of brownish-red color observed was evident, resulting in thesynthesization of silver nanoparticles.

Results: Brown discolouration represents formation of silver nanoparticles and the antioxidant activity was noted to be from 60% to 70% from 10 to 50µL. But when compared to standard vitamin C values the *Piper longum* silver nanoparticles showed less antioxidant activity.

Conclusion: The *Piper longum* mediated silver nanoparticles showed remarkable and considerable antioxidant activity but when compared with the standard sample values the antioxidant activity was low.

Key words: Antioxidant, Innovative technique, eco-friendly, green synthesis, *Piper longum*

Introduction:

paragraphs should be more concised and don't exceed 6 rows, references at end of paragraph and grouped in numerical pattern

Piper longum, a traditional plant used for regenerative medicine commonly known as the long pepper, is used to treat many pathological conditions (1). Various substitutes that are derived from Piper *longum*, one of the most important derivatives, is piperine. It's used to treat diarrhea, chronic bronchitis, stomach pain, asthma, hepatitis, and other respiratory insufficiencies. Advanced technology implying the use of nanoparticles in biomedical research, is gaining its agenda, incorporating almost all natural materials as sources (2).

Drug delivery is the most common use of nanoparticles in the current technological application. *Piper longum* is used as a source for obtaining the production of various nanoparticles and testing their efficacy. *Piper longum* possesses various pharmacologically beneficial activities, such as insecticide and acaricidal, anti-fungal, anti-amoebic, anti-asthmatic, anti-cancer, anti-oxidant, anti-inflammatory, antidepressants, antiulcer activities on routine consumption (3–5). The fruit of the *Piper longum* has hepatoprotective activity (6).

It is considered as an ayurvedic medicine providing enough treating ability to boost up the immune system to fight against various pathological disorders.Piperlongumine, sesamin, and sylvatin are important phytoconstituents of *Piper longum* (7). The chemical nature of these constituents is the ability to produce antioxidant activity. Nanoparticles are minute particles that range from 1 to 100 nm on a nanometer scale. Different nanoparticles possess different properties; they vary in physical and chemical nature.

Nanotechnology is now widely used for synthesizing drugs in the pharmaceutical industry. Formulation of nanoparticles, nanospheres, nano-capsules, nano-emulsion, and nanosuspensions are few applications of nanotechnology in the field of nanomedicine. The most common synthesized nanoparticles are plasmonic silver and gold because they possess unique physical and chemical properties when compared to other large surface areas and have strong electronic properties (8).

Nanoparticles are physically and chemically altered to have high potency to act upon the biological cell structure of the body (9,10). Silver nanoparticles are widely and commonly used for the conduction of electricity (11). They also have a localized resonance effect on the surface plasmon and have a wide range of antimicrobial activity against various microorganisms (12). The synthesis of silver nanoparticles involves 3 complex methods, they include physical synthesis, chemical synthesis, and biological synthesis (13).

The most important method used to synthesize silver nanoparticles involves the chemical method because of the nature of the silver nanoparticles which have the ease to simply dissolve in the prepared aqueous solution(14). Silver nanoparticles are commonly used in nanotechnology (15). Antioxidant activity is generally stated as prevention of oxidation of various constituents of the body, thus substances that tend to possess antioxidant activity which will block the oxidation of protein, DNA, and lipids present in ourbody.

An antioxidant prevents the formation of free radicals and their storage in tissues thus helps to prevent tissue depletion (16,17). Various naturally occurring plant sources and their products have antioxidant properties, which depresses and overcomes the oxidative stress caused by free radicals (18). The beneficial activities of *Piper longum* include antioxidant properties which have the ability to treat chronic conditions. Our team has extensive knowledge and research experience that has translated into high quality publications 19,20,21,22,23,24,25,26,27,28.29,30,31,32,33,34,35,36,37,38(numerical arrangement).

The aim of this study is to green synthesize and characterize the silver nanoparticles using *Piper longum* plant extract and to observe its antioxidant capacity.

Materials and methods:

Preparation of plant extract:

Commercially available dry powder of *Piper longum* was used for this experiment. This experiment was an intro vitro study which was conducted in Saveetha Dental College, Chennai, Tamil nadu. The experiment was carried out by dissolving 1g of *Piper longum* powder in 100ml of water. The moisture was then boiled in a heating mantle at 70 degrees Celsius for up to 10 minutes. The boiled mixture was then filtered using a Whattman number 1 filter paper to obtain the plant extract. Then 40 ml of plant extract was measured using a measuring cylinder and the mixture was added to 60ml of 1 mM silver nitrate (0.0169g) dissolved in 60ml distilled water. The lab technician reconfirmed the synthesized *Piper longum* silver nanoparticles to avoid sampling error for further analysis. The further analysis was that the formation of brownish-red color observed was evident, resulting in the synthetization of silver nanoparticles.

Reagent and Chemicals :

1g of *Piper longum* powder

1 mM silver nitrate (0.0169g)

2-10µg/ml concentration of *Piper longum* extract at various concentrations (10µL, 20µL, 30µL, 40µL, and 50µL)

0.1 mM DPPH in methanol

50 mM of tris HCl buffer

Control: BHT: Butylated hydroxytoluene

Antioxidant activity:

Piper longum was the plant used to determine the antioxidant activity by taking only 5 samples in distinct test tubes and adding 10µL, 20µL, 30µL, 40µL, and 50µL of the solution respectively using a micropipette. The micropipette usage was dealt with care to avoid methodology error. Random sampling method was performed to eliminate sampling bias and the validation of the procedure was done by principal investigator and by experts in nanotechnology. Other plants and activities were not tested in this present study.

To check the antioxidant activity, a DPPH assay was used to test the antioxidant activity of biogenic synthesized silver nitrate nanoparticles. Diverse concentrations (2-10µg/ml) of

Piper longum leaf extract interceded silver nitrate nanoparticle was mixed with 1 ml of 0.1 mMDPPH in methanol and 450µl of 50mM of tris HCl buffer(pH7.4)and incubated for30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. The percentage of inhibition was determined from the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The percentage of Inhibition was then calculated and correlated with the standard vitamin C concentration values which were not performed. And the antioxidant activity was correlated using Spearman's correlation in SPSS software version 23.0, the correlation graph was then represented and the p value was less than 0.05 for the non-parametric correlation analysis proving statistical significance.

Results: correct yellow coloured words as shown, paragraphs more concised ,figures and tables at end of paragraph, figure 5 mentioned below wasn't mentioned in introduction of results
The optical observation of the experiment was determined using a UV-visible spectrum providing the characterization of the prepared silver particles. The formation and synthesis of silver nanoparticles were determined by the brownish-yellow discoloration shown (**Figure1)**. The corresponding formation of silver nanoparticles with peaks at 517nm in UV spectrophotometer graph**(Figure 4)**. The presence of the surface plasmon resonance was the reason for the color change of the solution.

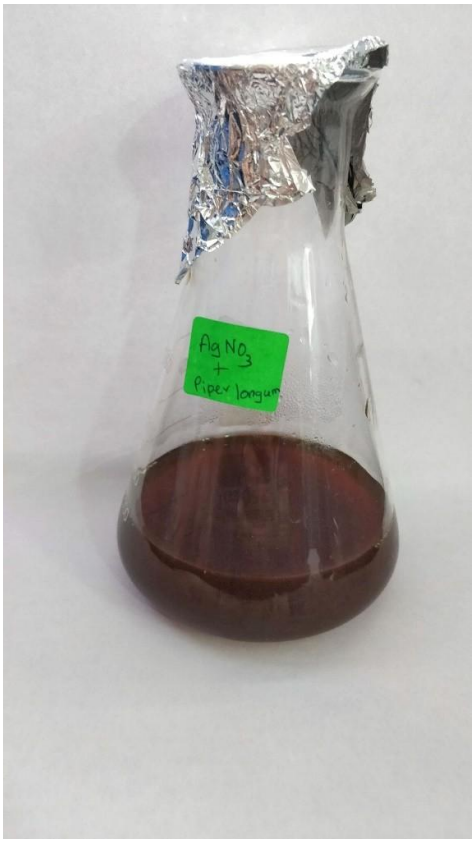

Figure 1: The color change indicates the synthesis of silver nanoparticles after 72hours and its corresponding correlation of the UV spectroscopy (figure 4).

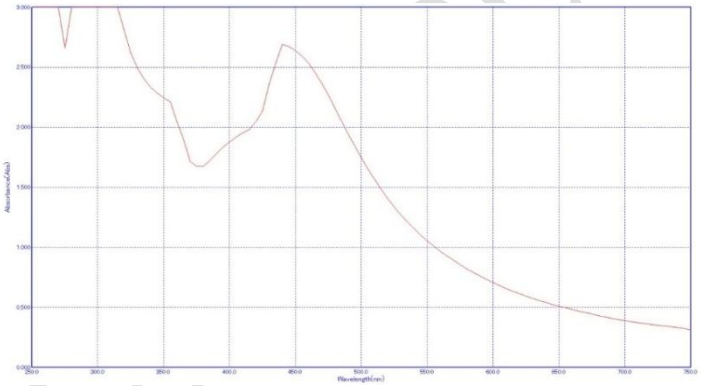

Figure 2: The UV/Visible spectrum of the silver nanoparticles synthesized using the *Piper longum* plant leaf extract.

Silver nanoparticles were formed by the reaction between the plant extract and the silver ions. Only potential plants were able to synthesize nanoparticles thus the change in color of the solution acted as a primary identification tool. The DPPH assay performed to test the antioxidant activity of the synthesized *Piper longum* silver nanoparticles was shown (**Figure2**).

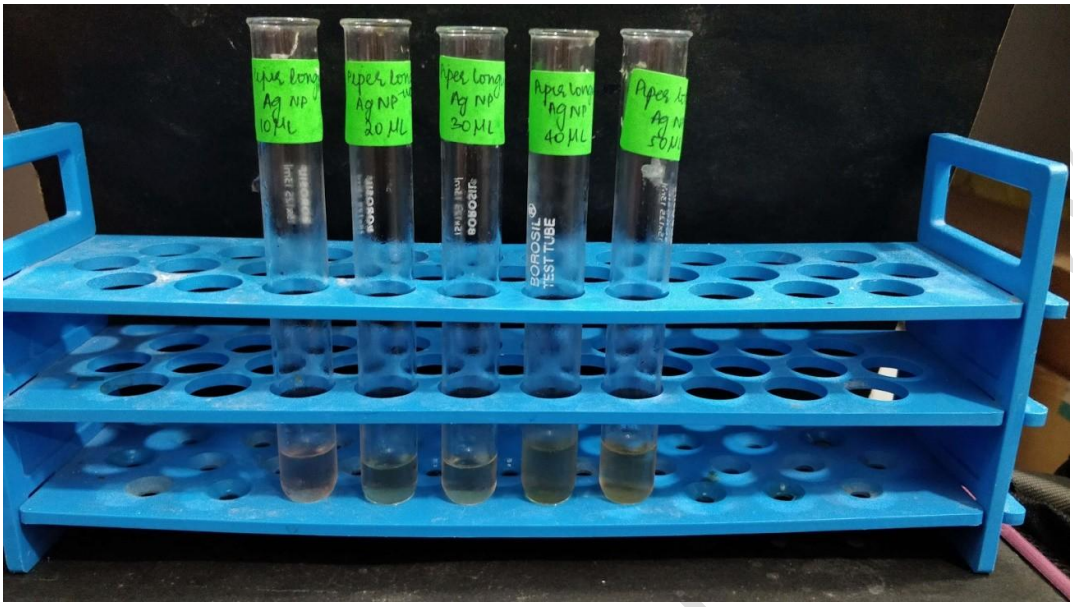

Figure 3: Antioxidant activity of the silver nanoparticles using the plant extract and the colour change is noted.

The percentage of inhibition of the synthesized *Piper longum* silver nanoparticles was noted to be 61.2%, 64.5%, 66.1%, 67.4% and 69.3% at 10µL, 20µL, 30µL, 40µL and 50µL respectively and whereas the values of the standard vitamin C solution are 76.5%, 78.8%, 84.9%, 89.2% and 92.3% at 10µL, 20µL, 30µL, 40µL and 50µL respectively(**Table 1**). The correlation analysis performed using SPSS version 23.0 and wasshown in (**Figure 5**). There was a positive correlation between the concentration of the sample prepared and percentage of inhibition.

Thus, the antioxidant activity wasincreasing with increased concentration of the synthesized silver nanoparticles**(Figure 3)**

Percentage of Inhibition (%)		
Concentration (µL)	**Silver nitrate nanoparticles**	**Standard**
10µL	61.2%	76.5%
20µL	64.5%	78.8%
30µL	66.1%	84.9%
40µL	67.4%	89.2%
50µL	69.3%	92.3%

Table 1: The Percentage of Inhibition for the different concentrations of the silver nanoparticles (ranging from 10µL to 50µL) in comparison with thestandard.

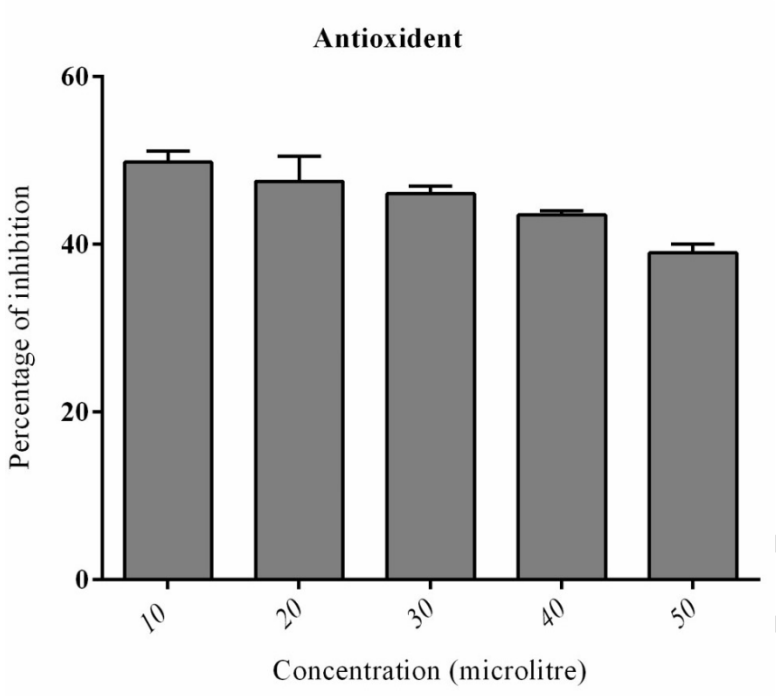

Figure 4: Antioxidant activity of silver nanoparticles. The X-axis represents the concentration of the silver nanoparticles and standard solution and the Y-axis represents the percentage of inhibition. Graph representing the percentage of inhibition observed with the different concentrations ranging from 10µL to 50µL in comparison to the standard value, the correlation was found to be positive using spearman's correlation. And the non-parametric correlation was found with *P* value less than .05 proving statisticallysignificance.

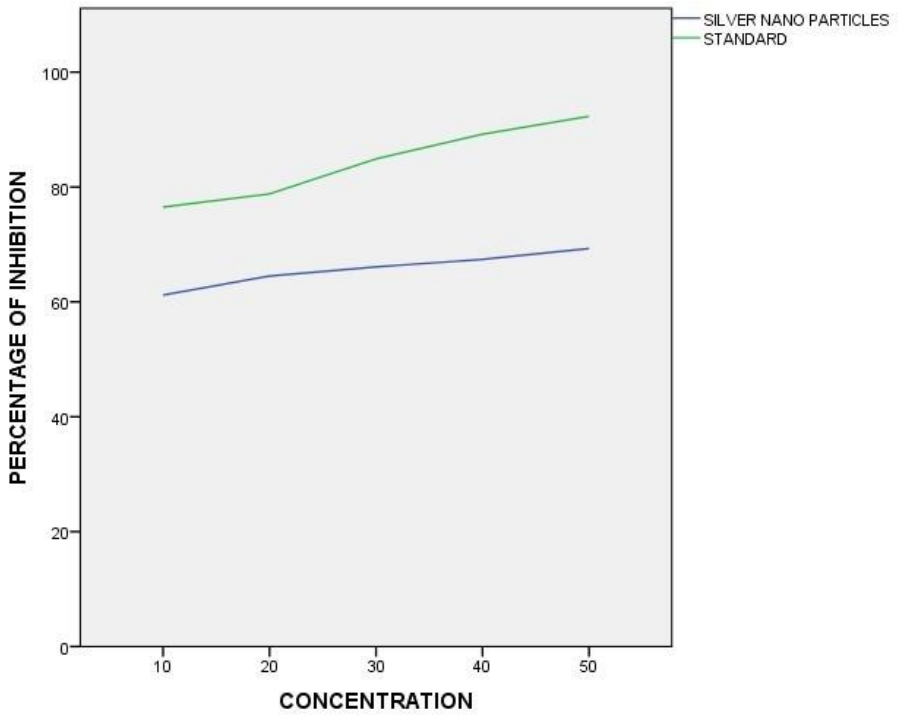

Figure 5: The line graph represents the comparative observation of the antioxidant activity of *Piper longum*AgNPs (blue) and the standard solution antioxidant activity (green). The X-axis represents the concentration of the silver nanoparticles and standard solution and the Y-axis represents the percentage of inhibition. The correlation was found to be positive using spearman's correlation. And the non-parametric correlation was found with *P* value less than .05 proving statisticallysignificance.

Discussion: correct yellow coloured verbs in past simple as shown, references at end of paragraph ,paragraphs no more than 6 rows

The present study investigated the optical observation which was the initial and novel technique that revealed the characterization of the synthesized silver nanoparticles as it presented a result of the UV- visible absorption spectra. The brownish-yellow discolorationindicated the formation of the silver nanoparticles. The silver nanoparticle formation showed significant color change because of the occurrence of the surface plasmon resonance (39-40). As the color change served as a primary tool, this was useful in the detection of the potential plants for the synthesis of silver nanoparticles.

The color change in the prepared solution was correlated with its UV spectrophotometer graph, in which the peak of the synthesized *Piper longum* solution with silver nanoparticles production is 517 nm. Similar studies showed that the prepared nanoparticles accompanied with the respective natural plant components used in assessing its antioxidant activity(41-42). The production of the chosen nanoparticleswere formed at the range of 400-520 nm in a UVvisible spectrophotometer graph(43). So compatible values of the UV spectrophotometer graph revealed the silver nanoparticle produced in 48 hrs.

2,2-diphenyl-1-picrylhydrazyl is an organic chemical compound which stands as the full form of DPPH, it is an assay performed to determine the antioxidant that is the free radical scavenging activity of a substance (44). So, the DPPH assay was performed to observe the antioxidant activity of the synthesized silver nanoparticles in this present study. A bar graph comparison represented the percentage of inhibition between the silver nanoparticles synthesized and the standard solution and a line graph version of the already displayed bar graph of the results were elicited.

In comparison to various works conducted by different authors, the study conducted by S.N Kharat etal., (45) the DPPH assay scavenging ability was in a purely dose-dependent manner, the scavenging property at 50µL was 15.23% which was the lowest concentration whereas when compared to the present study the antioxidant activity was 61.2% at the lowest concentration that is at 10µL which represents a correlation analysis.

The work conducted by Rajeshkumar (39)also concluded that the free radical scavenging activity was concentration dependent as well. Keshari et al., (46) concluded in study that AgNPshad the scavenging property of more than 29% stating its high free radical scavenging activity. Comparing works of DPPH radical scavenging with the work done by Zdenka et al., (47)stated thatthe activity was more than 89% at the highest concentration being 50µL.

Opposing work conducted by Chaudhary SK et al., (48) withoutthe involvement of nanoparticles showed that there was potential antioxidant activity exhibited by the synthesized *Piper longum* extract. The *Piper* species were accountable and known for their reduction of oxidative stress which made them a good source of an antioxidant substance. They were successfully used to treat Alzheimer's disease and Parkinson's disease which are common disorders affecting the central nervous system (49).

Generally the silver nanoparticles were remarkable for its significant antioxidant activity(50), they also showed numerous beneficial activities such as antimicrobial (51-52) and anti-inflammatory (53), so silver nanoparticles were most commonly used metal nanoparticles in nanomedicine (54). So silver nanoparticles played a crucial role in nanomedicine involving nano technological advances and also for biomedicine using genetic engineering (55). The use of silver nanoparticles made the present study economical, less cost effective, and easily available (56).

But not so on the bright side, recent researchers had found that silver nanoparticles consumption had caused some adverse effects on human metabolism. The toxic adverse effects included suppressing activity of the cell growth, division and multiplication (57), which was found to be in a pure concentrationconsumption and exposure manner.

The present study, was based on the silver nanoparticles having the property of possessing equally accessible antioxidant activity when compared to the standard scavenging property of the vitamin C solution (58). Vitamin C was chosen for this present study from the other two free radical scavenging vitamins that are A and E because of the slightly higher values of the antioxidant activities shown by them on various biomedical facilities (59).

Vitamin C free radical scavenging property is due to its capacity to donate an electron which provided the basic properties of a vitamin which was potent enough to show strong antioxidant activity. An antioxidant thus work by preventing the oxidation of the cells and tissues present in the human body and thereby neutralizing the free radicals produced (60). Substances which possessed strong antioxidant activity had a great potential to treat and cure many highriskhuman metabolic problems like atherosclerosis and cardiovascular disorders (61). In vitro studies have clearly ruled out the antioxidant activity of vitamin C in biological systems (62).

The present study,dealt with the comparison of the prepared *Piper longum* plant extract nanoparticles with standard Vitamin C values which showed significant values of free radical scavenging property. But the plant extract nanoparticle prepared had less antioxidant activity than the standard values. There were clear and accurate values of the antioxidant activity of the two solutions used to check its antioxidantactivity.

Limitations of the present study, were less sample size taken for the study, different plants could be taken as they all showed different antioxidant capacity and other beneficial properties. Only antioxidant activity of the *Piper longum* plant was assessed, and the specification in using silver nanoparticles. Value changes may be formed due to errors in experimentperformance.

According to the present study conducted, the free radical scavenging property of the prepared *Piper longum* plant extract silver nanoparticles was from an average range of 60% to 70% of the antioxidant activity. The maximum antioxidant activity was shown at the highest concentration that is at 50µL it showed nearly 70% of inhibition. Thus, the prepared *Piper longum* silver nanoparticles had good antioxidant activity and can be potentially used in nanomedicine to treat various biological conditions.

The future scope of this study, was the properties of synthesizing nanoparticles which were further implied in nanotechnology which may be useful for nanomedicine, biomedicine and other sourcing fields. Furthermore, research works were needed to potentiate the various aspects of the use of nanoparticles in different fields.

Conclusion: **correct yellow coloured verbs in past simple as shown,**

The *Piper longum* mediated silver nanoparticles showed remarkable andconsiderable antioxidant activity when compared with the standard values of vitamin C. The potential free radical scavenging property of the plant leaves extract mediated silver nanoparticles, was applicable in nanomedicine through the help of advanced technologies to support the medical voided fields. The properties of the synthesized nanoparticles were further implied in nanotechnology which may be useful for other sourcing fields. Further research studies were required to assess the full potential of the plant mediated nanoparticles.

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COMPETING INTERESTS

The author declares that there were no conflicts of interests in the present study.

AUTHORS' CONTRIBUTIONS

Taanya Imtiaz and Dr. S. Rajeshkumar prepared the *Piper longum* Ag nanoparticle solution and performed the DPPH assay to assess the anti-oxidant activity of *Piper longum*.

The data collection was done by Taanya Imtiaz. Taanya Imtiaz wrote the manuscript and it was edited and revised by Dr. R. Priyadharshini.

Dr. R. Priyadharshini and Dr. S. Rajeshkumar approved the submission of the manuscript.

ETHICAL APPROVAL

Nil

References:et al is added after 3 references as shown below

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